THE MIGRATION OF THE PRIMARY SEX-CELLS OF FUNDULUS HETEROCLITUS.

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The origin of the primary sex-cells in vertebrates is a problem which has received considerable attention during late years. Extensive summaries of the literature upon this subject may be found in the articles of Allen, 1911, and Jordan, 1917. Since no complete agreement with regard to details has as yet been reached, it is perhaps desirable to review briefly the earlier investigations, and to point out any discrepancies in the results already obtained which would seem to require further study. It would seem that more evidence is necessary to warrant safe conclusions on the matter.

Waldeyer (1870) first described the differentiation of the sex-cells from the "germinal epithelium" of a four-day chick. His view of sex-cell origin from the mesothelium covering the mesonephros was accepted at the time, and has been supported even by recent investigators. In 1880 Nussbaum advanced a rival theory as a result of his observations on the embryology of the trout and frog. He held that the sex-cells were of blastomeric origin, and further that there was an extra-regional segregation and a migration to the germ gland. Weismann (1886) popularized this idea in his work on the "continuity of the germ plasm."

Since the time of Nussbaum the evidence against the "germinal epithelium" idea has steadily increased. A number of investigators (Hoffman, 1892; Eigenmann, 1892; Beard, 1900; Woods, 1902; Allen, 1906, 1907, 1911; Dodds, 1910; Swift, 1914, 1915, 1916; Jordan, 1917) have failed to find any conditions not in accord with Nussbaum's theory.

However other recent workers (Firket, 1914, 1920; von Berenberg-Gossler, 1914) have been unable to accept this interpretation of the activities of these cells. According to their viewpoint, the

migration of the primary sex-cells is reduced to a mere phylogenetic vestige and is without any great genetic significance. Firket speaks of the primordial germ-cells as "primary genital cells" which, after migration disintegrate in the germ gland, being replaced by the true of "secondary genital cells" which arise from the peritoneal cells of the germ gland. Von Berenberg-Gossler regards them as mesodermal wandering cells of late endodermal origin, and describes them as contributory in the formation of the Wolffian ducts.

In our study of this general problem in *Fundulus* a number of questions have arisen as separate phases of the matter. The blastomeric origin of the sex-cells, their path and method of migration, and their history after reaching the germ gland are all matters requiring separate study. This paper has, as its special aim, the definite identification of the primary sex-cells and the determination of the germinal path in *Fundulus* embryos; that is, it is concerned with the second question listed. As yet our data upon the first question is inadequate and we have not enough material for a study of the third.

Material and Methods.

The material for this investigation consisted of the eggs of the teleost, *Fundulus heteroclitus* and was collected at Woods Hole in the summer of 1919. Care was exercised to insure an approximately uniform fertilization of the ova by mixing them with chopped testis. Two extensive series were preserved during the summer. Although accurate records were kept as to the age of each group, they are of only nominal value in this investigation, since environmental and individual differences cause variations in development of embryos of like age.

All embryos in these two series were fixed in Bouin's fluid and stained by the familiar "long method" for iron hæmatoxylin. Other material in various fixatives was also available for comparison. No trouble was experienced in obtaining slides which show clearly the cytological characteristics throughout the series as far as described. A majority of sections were cut 4 micra thick, but some were cut 5, 6 and 7 micra. The thickness of all sections

was of course recorded. Most of the observations were made from serial transverse sections because they show the dorso-ventral position of the sex-cells more clearly in relation to the outstanding features of the developing embryo than do those cut longitudinally.

Observations.

Criteria of the Primary Sex-cells.

The enumeration of criteria for any group of cells as distinguished from all others in a series of embryos is a task which promises but doubtful results. There can be no question however that the primary sex-cells do have distinctive characteristics which make them easily recognizable, during the resting stages, to one who has had them under observation. It is not always feasible positively to identify the cells during division.

Throughout the migration period these cells maintain the same general characteristics. There are, to be sure, slight variations in the ratio between the nuclear and cytoplasmic elements, in size and in the character and arrangement of the chromatin granules; but these features may be observed only on close inspection, rather than in a preliminary study of the primary sex-cells.

The primary sex-cells vary in diameter from 9 to 12 8 micra. As contrasted with other cells they are spherical or ovoid with very definite cell outlines. The nuclei conform to the general shape of the cell body within which they are located. The cytoplasmic content is always clearer and takes less stain than that of the surrounding cells. Likewise the achromatin of the nuclei is quite clear, allowing the chromatic granules to stand out in bold contrast. The linin network is directly beneath the nuclear membrane, and due to this arrangement the chromatin granules are distributed peripherally over the nucleus. This peripheral arrangement of the chromatin is a constant distinguishing characteristic not to be mistaken, for it is never produced in any other cells. The linin network is connected to one, or more frequently to two nucleoli which are located near the center of the nucleus. No peculiar invagination of the nuclear membrane, such as was reported by Dodds (1910), was observed in *Fundulus*. An unusually large centrosome is, as a rule plainly visible in the cyto-

plasm. In older embryos these cells may be recognized by their size, since they are larger than any others which may occur in the same region.

Figures 1, 2, 3 and 3*a* are surface views of typical sex-cells. The peripheral arrangement of the chromatin has been emphasized in drawing Fig. 4*b*, by focusing upon a level with the center of the nucleus. Fig. 4*a* was obtained by focusing higher on the surface of the same nucleus. Thus Fig. 4*b* represents the chromatin knots in an optical section; while Fig. 4*a* shows them in a surface view. If Fig. 4*a* were superimposed upon Fig. 4*b* the resulting composite would be a cell not unlike that represented in Fig. 3, except that in the latter the knots have taken the familiar granular appearance.

A positive identification of the primary sex-cells was first made in a 24-day embryo. From this stage their path was followed backwards, through all the intermediate phases of migration, until they were no longer evident. It is considered expedient to describe their position in the 24-day stage, so that no question shall arise later as to the exact nature of the migrating cells whose course is to be traced.

Having established the identification of the sex-cells in the late embryo (24 days) their migration may be traced from their earliest appearance up to this stage. Although this sequence is contrary to our experimental procedure, it is believed to be more easily followed by the reader.

24-Day Embryo. 5.75 Mm. Long.—At the 24-day stage the sex-cells lie in the sac-like anlagen of the germ glands, which have formed dorsally and slightly laterally to the hind gut. Here they are unquestionably recognizable (Fig. 5). These cells are numerically inferior to the peritoneal cells which surround them and which are beginning to take a very active part in the formation of the future sex gland. The size and position of the germ gland anlagen in relation to the embryo is shown in Figs. 6 and 7. No attempt has been made to ascertain the average number of sex-cells which are present during this stage.

Whether these are the true sex-cells as maintained by many investigators, or whether they later disintegrate and become replaced by "secondary genital cells" as indicated by Firket (1914,

1920) and others, is a question which may be omitted from the present discussion.

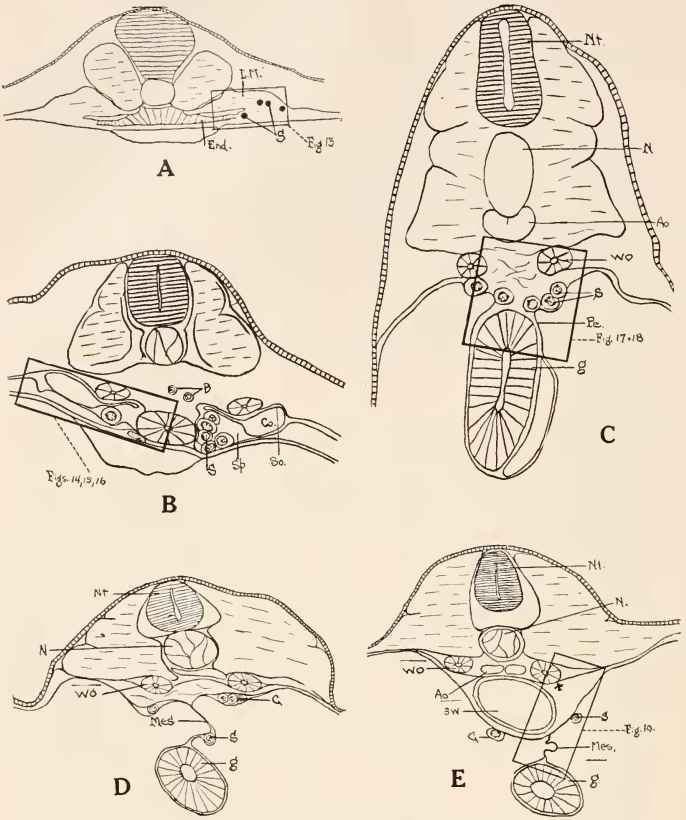
Observations of the Germinal Path.

From many available embryos the following were selected for consideration because they constitute representative stages, and are essential to a clear understanding of the migration of the primary sex-cells.

Embryos from 46 to 50 Hours.—Three embryos of this very early stage, designated in our material as B′ 21X, B′ 21L and B′ 23 respectively were carefully studied. Others were available but they were used merely as checks on the three which are reported.

The position of the sex-cells at this stage is most striking. There is a wide range of distribution in each embryo. The most anterior of the sex-cells were invariably farther along the germinal path than were the more posterior ones of the same embryo. To demonstrate this fact Fig. 20 has been drawn; it is an exact outline diagram of B′ 21X, reconstructed by the most accurate means possible. The lateral extent of the neural tube, of the mesoderm and the positions of the sex-cells were determined by measuring from the median line. The thickness of the sections was known. These determinations were plotted on millimeter paper and the outline filled in as indicated by the plotted guides. The exact antero-posterior positions of the sex-cells were determined by counting the sections of the serially sectioned embryo. Figs. 9, 10 and 11 are outline drawings from the embryo B′ 21X which was sectioned transversely, and Fig. 12 from B′ 21L which was sectioned longitudinally. These drawings show the exact positions of the sex-cells more clearly than would be possible in a written description.

The letters *A, B* and *C* on Fig. 20 indicate the positions of the sex-cells shown in Figs. 11, 10 and 9 respectively. Fig. 11 illustrates clearly the position of the primary sex-cell in the extra-embryonic region at the posterior of the embryo. Four sex-cells are shown in Fig. 12 as being lateral to the undifferentiated endodermal cell mass and ventral to the elongating tail. In Figs. 9 and 10 the migration has progressed proportionally to the devel-



TEXT FIG. *A*. Semidiagrammatic transection through the posterior of the 50-hour embryo B′ 23. The spots indicate the positions in which the greater portion of the sex-cells are found at this stage. The rectangle includes the area which is drawn in detail in Fig. 8. × 225.

TEXT FIG. *B*. Transection from the 105-hour embryo, showing the first decided advance over the stage illustrated in Text Fig. *A*. Here the gut, Wolffian ducts and the cœlome have taken form. The rectangle includes the area drawn in Figs. 14, 15 and 16. × 225.

TEXT FIG. *C*. Showing progress of development after 6 days. Figs. 17 and 18 are detailed drawings of the area included in the rectangle. × 225.

TEXT FIG. *D*. Transection through the developing gonads of the 9-day embryo, B 34. The sex-cells are collected ventral to the Wolffian ducts. The dorsal mesentery shows a decided change from conditions found in earlier stages. × 90.

TEXT FIG. *E*. From the 13-day embryo B 42, showing the effect of the developing swim bladder. × 90.

opment of the embryo at the regions represented. The sex-cells lie between the periblast and the endoderm in Fig. 10; while in Fig. 9 their position is below the mesoderm and lateral to the developing hind gut.

The positions of the most anterior sex-cells in embryo B′ 23 are indicated in Text Fig. *A*. The rectangle in this text figure includes the region which is drawn in detail in Fig. 8. Here a primary sex-cell is shown which is entirely free from any possible connection with the lateral mesoderm. It can scarcely be said to lie in, but rather lateral to the gut endoderm. It is half buried in the periblast. This fact suggests intimate relation with this nutritive layer. The cell figured is one of the few ever found with an irregular outline. This might seem to suggest amœboid activity, but this type is so extremely rare that it may be neglected from consideration.

Fig. 13 from B′ 23 shows a sex-cell which is .06 mm. to the rear of the one just mentioned. It is plainly in that portion of the lateral mesoderm which will develop into the splanchnic layer upon the formation of the cœlome (about the third day).

Observations of these early embryos show several important facts. The primary sex-cells are as truly characteristic and as easily recognizable as any found in the germ glands of later stages. They are located in the posterior half of the embryo, becoming gradually more numerous as the anterior part of this region is approached. Laterally they range from the extra-embryonic region to within the lateral mesoderm and the edge of the developing gut. In general their progress along the germinal path is directly proportional to the development of the embryo.

105-Hour Embryo.—Text Fig. *B* shows the relative positions of the sex-cells in the 105-hour embryo, B′ 26. As in previous cases the rectangle indicates the area from which Figs. 14, 15 and 16 were drawn. These three figures from the same embryo illustrate the full extent of the migration at this stage. On the left side of the embryo the sex-cells are found scattered all along the splanchnic mesoderm, from the region very near the split in the lateral mesoderm (Fig. 14) to that at the side of the gut (Fig. 16). On the right of Text Fig. *B* the sex-cells on the opposite side of the embryo are shown massed lateral to the gut. Should

the lateral mesoderm fuse above the gut, the formation of the dorsal mesentery would result and the position of the sex-cells would be identical to that found in later stages. Ex. Figs. 17 and 18.

6-Day Embryo. 2.6 Mm. Long.—Text Fig. *C* represents the position of the sex-cells as found in the 6-day embryo. At this time they are apparently in a state of rapid migration from the loose mesenchyme dorsal to the hind gut, to the positions ventral to the Wolffian ducts. Because of the laterally compressed condition of the embryo, which was due to the softness of the paraffin at the time of cutting, the transections are not exactly typical. However this embryo has been used since it represents most clearly the transitional stage between those figured in Text Fig. *B* and *D*. Figs. 17 and 18 are detailed drawings of the 6-day stage. They illustrate the complete extent of the migration in the mesentery. The majority of the sex-cells were in the dorso-ventral position indicated by the cells in Fig. 17, while only a few were in that shown in Fig. 18. The more anterior sex-cells were farther along in the germinal path (being nearer the Wolffian ducts) than the more posterior ones. The position of the germ gland anlagen ventral to the Wolffian ducts is illustrated in Text Fig. *D*.

13-Day Embryo. 4 Mm. Long.—Text Fig. *E* represents the position of the germ gland anlagen as found in the 13-day embryo B 42 (4 mm.). The rectangle in Text Fig. *E* includes the region which is drawn in detail in Fig. 19. Rarely more than one sex-cell is found at this stage in any one section of a germ gland anlage. The anlagen are little more than protuberances from the peritoneum, containing relatively few peritoneal cells (although the sex-cells are surrounded by them) and they have not yet reached the future position of the gonads. It is obvious that the sex-cells which are contained in the peritoneal sac are all pushed ventrally by the developing swim bladder. One cell was observed in the position indicated by the cross in Text Fig. *E*. It was not included in the peritoneal sac and seemed apparently helpless in the loose mesenchyme ventral to the Wolffian duct. This cell had been delayed in reaching this position, had not been included in the sac, and in consequence of this fact it had not been

influenced by the action of the swim bladder. One of these lost cells is shown in the mesentery in Text Fig. *D*. The future of such cells is an open question.

A count of the sex-cells in this embryo, B 42, gave 64. There was never any question of recognizing these cells, for no cells of doubtful character were observed. Four of these cells found were in the mesentery above the gut, and one was in the loose tissue ventral to the Wolffian duct. No cases of disintegrating sex-cells were observed in our *Fundulus* material, although such conditions are reported by some investigators.

The *24-day embryo* shows the next advance in the germinal path. This stage has been considered perviously in connection with "Criteria of the Primary Sex-Cells."

Table of Average Diameters.

For the purposes of this investigation the diameters in micra were found by averaging the long and short dimensions of the cell and nucleus. By this method a number of representative sex-cells of embryos in all stages of migration were measured under the oil immersion and the following results were obtained.

Embryo.								Average.
1.B'21X (embryonic region)								
46 hours	Cell	10.0	10.5	9.6	10.4			10.1
	Nucleus	6.0	6.4	5.6	5.2			5.8
2.B'21X (extra-embryonic)								
46 hours	Cell	12.8	11.2	11.2	11.5			11.7
	Nucleus	6.0	6.1	6.5	6.5			6.3
3.B'26 105 hours	Cell	9.9	11.0	10.7	10.4	10.2		10.4
	Nucleus	6.1	6.2	6.2	6.1	5.8		6.1
4.B 30 6 days	Cell	12.3	12.4	12.7	11.2			12.2
	Nucleus	6.6	6.6	7.0	6.2			6.6
5.B 42 13 days	Cell	11.5	11.5	11.9	10.7	9.9	11.5	11.2
	Nucleus	7.2	6.6	8.0	7.8	5.7	6.3	6.9
6.B 65 24 days	Cell	11.5	11.8	10.2	10.4	9.0	9.5	10.4
	Nucleus	6.1	7.8	7.3	7.0	6.2	6.6	7.2

Multiplication of the Sex-cells.

The early distribution of the sex-cells (Figs. 20 and 21) is best explained, we believe, in connection with the streaming of the organ-forming substances which contribute materials to the embryo body. In most processes of this nature not only cell trans-

portation but cell division takes part. Certain workers with other forms have held that the movement of the cells into the anlagen of the gonads is not the only factor responsible for their increase, but that multiplication actually occurs during the period of translocation. Mitotic figures have never been observed in *Fundulus* among the recognizable sex-cells which are within the embryo, although a most thorough search has been made for them in many embryos at all stages of development. A count of these cells in several specimens in various stages reveals the fact that there is a tendency for their number to vary more or less from the average established (67). However there is not enough variation to convince one that there is any marked multiplication of the sex-cells during the migration period. These facts naturally lead to the conclusion that the first period of multiplication takes place in the extra-embryonic region.

In the description of the earliest embryos referred to in this report and in the figures presented, emphasis has been placed upon the fact that the primary sex-cells in any one embryo are not in the same phase of migration. Furthermore observations upon all stages show that development becomes more advanced anteriorly than posteriorly. It is of further interest that in embryos containing sex-cells both within and without the body, the number falls below the average for older stages. These conditions and the fact that no sex-cells have been found in any region other than that already described, suggest the explanation that these cells multiply in the extra-embryonic region. Indeed the four sex-cells illustrated in Fig. 12 may indicate recent cell division by their very association. If they are not of recent and identical origin they would probably be farther separated than they are in this figure. These views are presented only tentatively, due to lack of sufficient material to warrant definite statements on this multiplication; for no mitotic figures have ever been seen in the extra-embryonic region to substantiate this belief in a division as suggested. Our material has not permitted a careful study of this matter. However considering the longitudinal distribution of the sex-cells in the earliest available embryos, and their tendency to approach a common average number in each individual, one is inclined to regard them as being of unquestionably

earlier origin than it has been possible thus far to trace them. It seems not unreasonable to believe that the fore-runners of these cells have been segregated at a time very early in the development of the germ ring.

Discussion and Conclusions.

This paper attempts to identify definitely the sex-cells which are present in the 24-day embryo as the "primordial germ cells" of previous writers, or as the "primary genital cells" of Firket. It also presents evidence on the manner in which these cells reach their final destination.

The method of embryo formation in the teleosts has a bearing upon the question of sex-cell migration in *Fundulus*. It will be recalled that the anterior portion of the embryo is formed from the head fold, which may perhaps be nothing more than a thickening on the germ ring; while the body or posterior portion is to be regarded as the result of the developmental process termed concrescence. It is only this latter portion of the body that is involved in the formation of the sex-cells. The eggs have a large amount of yolk, and a very distinct germ ring. As cell proliferation takes place, the germ ring moves gradually downward over the yoke mass. The primitive streak moves backward and receives the converging limbs of the germ ring posteriorly. The material of the halves of the germ ring, after fusion, is differentiated into the embryo posterior to the head process. The rudiments of the embryo body are not clearly marked out in *Fundulus* until the germ ring is completely closed.

The earliest primary sex-cells which we have located are from embryos in which the germ ring has been closed but a few hours, and in which the tail is just beginning to elongate. Their position in the extra-embryonic region lateral to the undifferentiated endodermal cell mass at the posterior half of the embryo is indicated in Fig. 20. In other embryos of the same stage of development, numerous primary sex-cells are present in practically the identical relation to the embryo that is clearly demonstrated in Fig. 20. These sex-cells invariably lie just above the periblast and are associated with the sheet of cells which is a lateral expansion of the undifferentiated endodermal cell mass

(peripheral endoderm, Allen). The complete germinal path from this position to one lateral to the hind gut may be followed in almost any embryo of from 46 to 50 hours. This very advantageous condition is made possible by the greater development near the middle of the embryo, for it is only a natural result of embryo formation by concrescence that development is progressively greater anteriorly from the point of convergence of the germ ring.

These cells are transported from the edge of the embryonic region medially, to positions just beneath or within the endodermal cell mass, as the case may be. They are carried passively from one position to another by the same forces of growth which bring together the halves of the germ ring. The influence of this factor can scarcely be over emphasized. Although not outwardly as apparent as in earlier stages, these forces are nevertheless responsible for the flowing of the streams of embryonic material towards the future position of the organs which are to develop therefrom.

The sex-cells come to lie within these shifting layers of embryonic endoderm and mesoderm and naturally accompany these layers in their changes of position. Because of the fact that the movement of the sex-cells is not active but rather dependent upon that of surrounding layers, the expression "migration" seems rather unfortunate. Some term such as "translocation" would perhaps be more truly expressive of the actual conditions.

These cells come to lie in the edge of the embryonic region, and when a portion of the undifferentiated cell mass gives rise to gut endoderm and another to lateral mesoderm, they follow one layer or the other. The sex-cells follow one or the other of these layers until they reach a position lateral to the newly formed gut. Which layer is chosen apparently depends upon chance. Those cells which have been carried in the edge of the endoderm never enter the gut, but move dorsally from the side of it into the lateral mesoderm. Here they join the sex-cells which have been carried in the mesoderm. By this time the split, resulting in the formation of the cœlome between the splanchnic and somatic mesoderm has taken place. Although the sex-cells are associated with all parts of the lateral mesoderm before the forma-

tion of the cœlome, it is a noteworthy fact that they never occur within the somatic layer after differentiation.

From this position lateral to the hind gut the cells are in the general dorsal movement of the mesoderm which eventually results in the formation of the intestinal mesentery. The cells from either half of the embryo remain apart and seem to lie in separate streams of mesodermal cells which are flowing toward the Wolffian ducts. But although there may be a pause here, at no time do the sex-cells appear to establish any intimate relation with the cells of these ducts (Text Fig. *D*). From the evidence at hand, an explanation of the function of these cells which makes them contributory to the development of the already well-formed Wolffian ducts, as suggested by certain investigators, does not seem plausible in *Fundulus*.

As the sex-cells reach a position nearly ventral to the Wolffian ducts they become surrounded by a single layer of peritoneal cells. This covering develops until the position of the future sex organs is attained; the sex-cells then rest in sac-like protuberances from the peritoneum, the germ gland anlagen. Assisting in the movement which brings the sex-cells into their future positions, are several factors entirely external to the germ glands. For example, there is a rapid proliferation of the loose mesenchyme dorsal to the gut and the development of the swim bladder which results in a median down pushing. The ventral movement (Text Figs. *D* and *E*) from the region of the Wolffian ducts is clearly due to the wedge-like effect produced by the growing swim bladder. That this process is necessarily passive is evident from the fact that amœboid activity of the cells included within the germ gland would be unable to produce any change in its position.

From the evidence in *Fundulus* it is apparent that the sex-cells enter the embryo and are located in the germ glands by the same forces that are influential in the distribution of the other organ forming substances of the body. Their "migration" is not to be looked upon as different from that of any other group of cells. But while the sex-cells are not amœboid, there is nevertheless reason for misunderstandings which have arisen regarding their activities. In the first place they are relatively few in compari-

son to the great numbers of cells in the surrounding tissues. Although the entire mass of cells is continuously in motion, only the movement of the sex-cells is at all noticeable. They are shifted about by the active surrounding tissues and naturally assume slightly irregular outlines at times, due to the unequal tension upon the cell membrane. Through a misinterpretation of the conditions within the embryo these sex-cells may easily be accredited with peculiar powers of locomotion. A sex-cell, as a slowly drifting cloud, can be seen gradually to change its position; but movements of the tissue cells about it, due to their location in continuous layers, are so inconspicuous as to go unnoticed. Because of this, the movement of the sex-cells should be considered merely as the passive indication of the rate and direction of progress of contiguous layers.

Summary.

1. The earliest primary sex-cells found in *Fundulus* were located in the peripheral endoderm, lateral to the posterior half of the 46-hour embryo. No sex-cells were observed in that part of the embryo which develops from the head fold.

2. The germinal path leads from the peripheral endoderm, into the border of the undifferentiated endodermal cell mass. When this cell mass splits to form gut endoderm and lateral mesoderm, the sex-cells proceed medially with either layer. By the time the gut is formed, these cells are lateral to it; they all eventually become located in the splanchnic mesoderm of this region. From here the sex-cells migrate dorsal to the hind gut, thence to the region ventral to the Wolffian ducts. Here they become surrounded by peritoneal cells which form the somatic portion of the gonads. From this position the germ gland anlagen are shifted back to their final location dorsal to the gut.

3. There is very little multiplication of the sex-cells during the period of migration. Division apparently takes place in the extra-embryonic area, and is not renewed to any marked extent until after the sex-cells become located in the germ glands.

4. The constant distinguishing characteristics insure positive identification of these cells throughout all phases of their migration, and leave no reason to question their identity as being the "primordial germ cells" of previous writers.

5. Migration is passive, being due to forces of growth which are altogether external to the cells themselves. These forces of growth are factors common to the development of the organs formed in the body of the teleost embryo.

6. Evidence derived from this study of *Fundulus* is an absolute harmony with the theory of early segregation of these primary sex-cells.

Note

Some time after the manuscript of this paper had been sent to the press, an extensive article by Okkelberg, entitled, "The Early History of the Germ Cells in the Brook Lamprey, *Entosphenus wilderi* (Gage), up to and Including the Period of Sex Differentiation," appeared (*Jour. Morph.*, Vol. 35, No. 1, 1921). This article contains much data and many important conclusions, and it is to be noted (on pages 35 and 36) that the author, in considering the sex cells, has discussed their methods of migration. It is of great interest that the conclusions reached by Okkelberg on this matter for the lamprey are very similar to our own upon *Fundulus*.

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EXPLANATION OF ILLUSTRATIONS.

All figures in this report were drawn with the aid of a camera lucida. Any lens combinations which were considered necessary to produce the best results were used. The magnification as given for each figure was calculated carefully and is correct for the reproductions as they appear on these plates.

ABBREVIATIONS.

a., anus.
Ao., aorta.
B., blood cells.
c., centrosome.
cr., chromatin knots.
Co., cœlome.
Ect., ectoderm.
E.M., endodermal cell mass.
En., gut endoderm.
G., germ gland anlagen.
g., gut.
L.M., lateral mesoderm.
li., linin network.
M., mesoderm.
Mes., gut mesentery.
N, notochord.
Nt., neural tube.
nu., nucleolus.
P., peritoneum.
pe., periblast.
P.N., periblast nucleus.
S., sex-cell.
sw., swim bladder.
So., somatic mesoderm.
Sp., splanchnic mesoderm
T., elongating tail.
Wo., Wolffian duct.

DESCRIPTION OF ILLUSTRATIONS.

PLATE I.

FIG. 1. A typical primary sex-cell from a 105-hour embryo (B′ 26). Showing the large centrosome, two nucleoli and chromatin knots scattered over the periphery of the nucleus. × 1420.

FIG. 2. A typical sex-cell from a 6-day embryo (B 30). Chromatin granules finer than in the preceding cell. × 1420.

FIG. 3. Sex-cell from a 100-hour embryo (B′ 25–3). From the extra-embryonic region. It is closely associated with the periblast. In other sections the peripheral endoderm may be seen out over this area. × 1420.

FIG. 3*a*. Sex-cell from a 9-day embryo (B 34). Chromatin arrangement is intermediate between that found in Figs. 1 and 2. × 1420.

FIG. 4*a*. Surface view of a nucleus from a sex-cell in a 105-hour embryo, showing the chromatin knots in surface view. × 1420.

FIG. 4*b*. From the same nucleus as the one used in Fig. 4*a*. Obtained by focusing upon the center of the nucleus, illustrating the chromatin knots in optical section. × 1420.

FIG. 5. Showing the sex-cells in the germ gland anlagen dorsal to the hind gut of a 24-day embryo. The function of the peritoneal cells at this stage is quite evident in this illustration. The sex-cells are completely surrounded by mesoderm. × 700.

FIG. 6. Transection through a 24-day embryo (B 65–2) taken at the position indicated by the plane X–X′ in Fig. 7. Showing the position of the gonads in relation to other parts of the embryo; especially as regards the developing swim bladder. × 120.

FIG. 7. Longitudinal section lateral to the median line of the 24-day embryo (B 65). Illustrating the position of the gonads as being the same as in the adult. Migration ceases at this point. The activities within the gonads after they have reached this point of development will not be considered at this time. × 25.

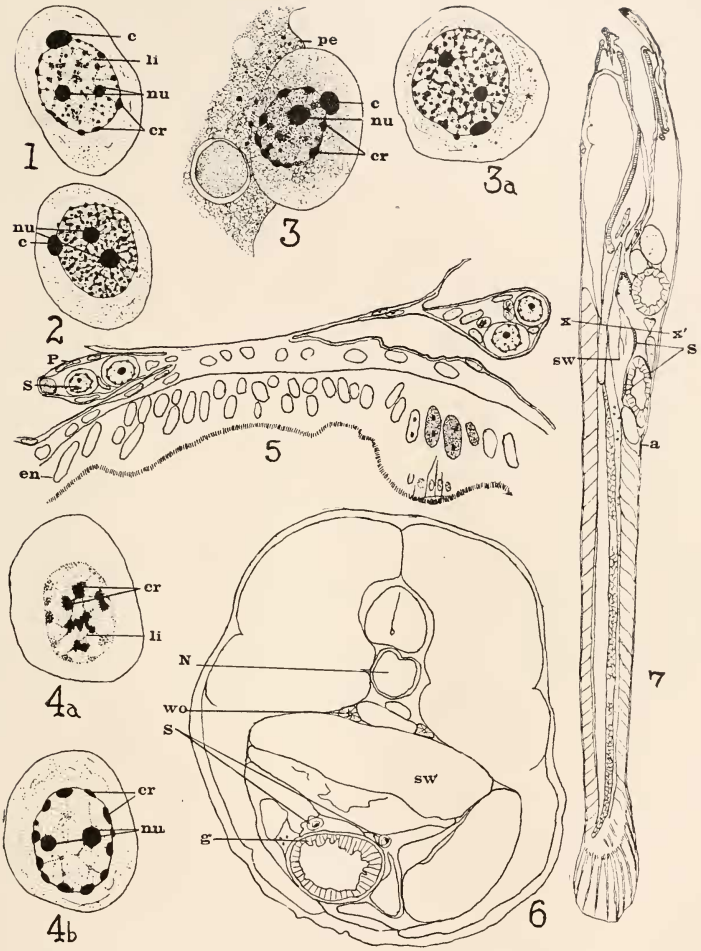
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Plate II.

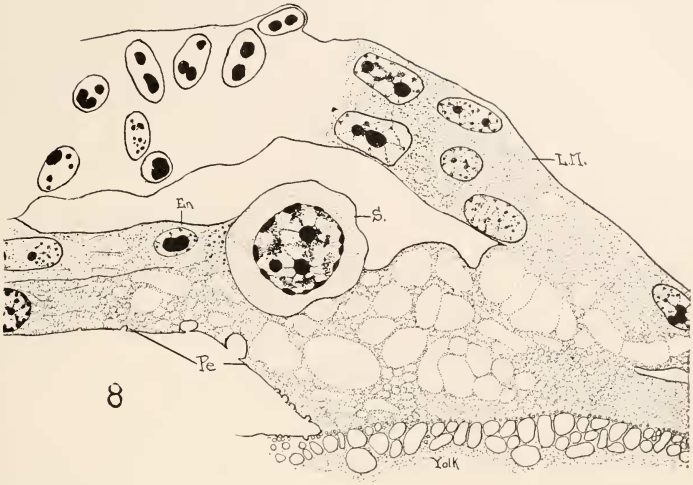
Fig. 8. A sex-cell from the 50-hour embryo (B′ 23). Showing in detail the region at the edge of the embryo, where the germ layers meet the periblast. The sex-cell is in the edge of the gut endoderm, entirely removed from any connection with the lateral mesoderm, and is partially imbedded in the periblast. Several very early cells have been found in this relation to the periblast, but so far it has been impossible to establish any significance to this fact. Due to exertion of unequal tension upon the cell membrane, the cell outline appears slightly irregular. × 1400.

Fig. 9. Semi-diagrammatic transection of the 46-hour embryo B′ 21 X taken at the position indicated by the line "*C*" in Fig. 20. At this early stage the lumen of the gut is not formed completely, even in this most anterior region. The sex-cells lie between the periblast and the lateral mesoderm, at the side of the developing hind gut. × 300. (The positions of the sex-cells in the germinal path correspond to certain stages in development of the gut.)

Fig. 10. Transection of embryo B′ 21 X taken at the position indicated by the line *B* in Fig. 20. Here the gut endoderm and the lateral mesoderm are differentiated to a certain extent, although the splitting of the endodermal cell mass has not yet occurred. The sex-cell lies above the periblast in the edge of the cell mass. × 300.

Fig. 11. Transection of embryo B′ 21 X taken at the line "*A*" in Fig. 20. The sex-cell illustrated is in the extra-embryonic region, associated closely with the peripheral endoderm. × 300.

Fig. 12. A longitudinal section through the elongating tail of the 46-hour embryo B′ 21 L. The 4 sex-cells illustrated are in the peripheral endoderm at the extreme posterior of the embryonic area. × 300.



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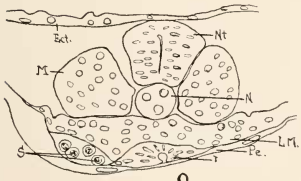

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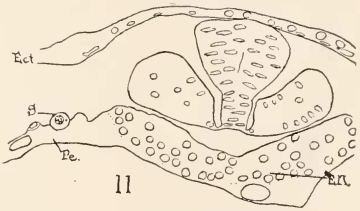

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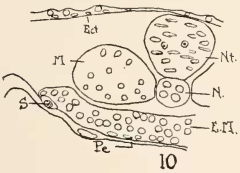

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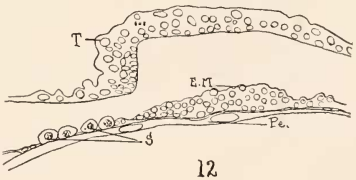

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Plate III.

Fig. 13. Sex-cell from the 50-hour embryo B′ 23. It lies in the extreme edge of the lateral mesoderm, just dorsal to its separation from the periblast. This cell lies in that portion of the mesoderm which will give rise to the splanchnic layer. × 700.

Fig. 14. A sex-cell in the splanchnic mesoderm of the 105-hour embryo B′ 26. The cell is just medial to the point of differentiation between the somatic and splanchnic layers. This stage also shows an advance over the one illustrated in Fig. 13, in that the gut and Wolffian duct have taken form. × 700.

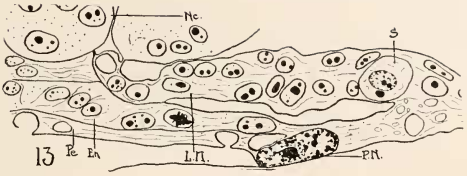
Fig. 15. A group of sex-cells from the region anterior to that drawn in Fig. 14. Here the sex-cells are approaching the side of the hind gut. × 700.

Fig. 16. A sex-cell from embryo B′ 26, in the region anterior to those illustrated in Figs. 14 and 15. The more medially placed cell is in the splanchnic mesoderm lateral to the gut. As this layer grows up over the gut to form the dorsal mesentery, the sex-cells will naturally be brought to lie in this region. × 700.

Fig. 17. Four sex-cells from the 6-day embryo B 30 in the mesentery dorsal to the gut. This shows in detail the region included in the rectangle in Text Fig. *C*. The extraordinary width of the mesentery at this stage is doubtless due in part to the presence of the sex-cells. × 420.

Fig. 18. This figure illustrates the extremes of the migration at this stage. No cell was found at any earlier stage than the one near the gut, none later than that ventral to the Wolffian duct. From embryo B 30. × 420.

Fig. 19. Showing the development of the gonads in the 13-day embryo B 42. The sex-cells are fixed in sac-like protuberances from the peritoneum. From this figure it is possible to obtain an idea of the effect produced by the rapid growth of the swim bladder, in literally pushing the gut and all related tissues ventrally. It is also interesting to observe that the peritoneal covering renders this cell dependent upon surrounding tissues for movement to its final position dorsal to the gut. × 420.



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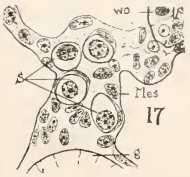

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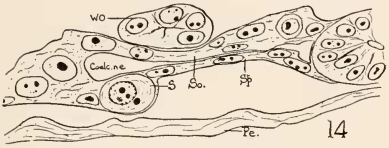

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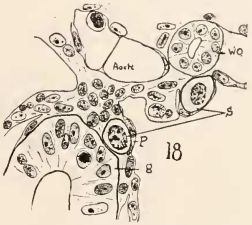

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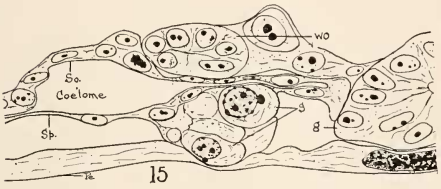

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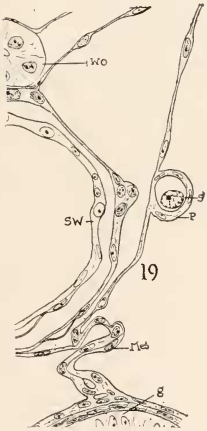

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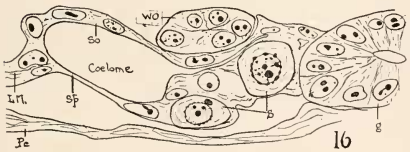

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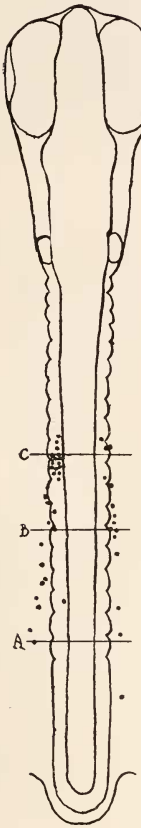
Plate IV.

Fig. 20. A diagrammatic reproduction of embryo B′ 21 (46 hours) demonstrating the distribution of the sex-cells at this early stage. The variation in anterior-posterior development is very noticeable and is explained fully in the text. × 65.

Fig. 21. Reproduction of embryo B′ 26 (105 hours) constructed similarly to Fig. 20. Showing the positions of the sex-cells as being more medially placed than in the earlier stage. The cells on the right are not as far along with migration as those on the left; the former group migrated with the mesoderm and the latter followed the gut endoderm. × 65.



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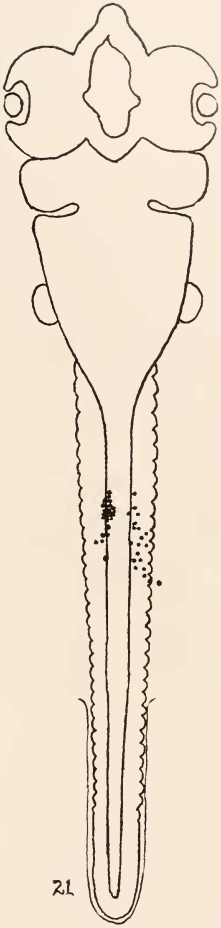

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A. RICHARDS AND J. T. THOMPSON.

SPERMATOGENESIS OF APHIDS; THE FATE OF THE SMALLER SECONDARY SPERMATOCYTE.

H. HONDA.

CONTENTS.

I. INTRODUCTION.

It has been shown by Morgan and von Baehr that in aphids the primary spermatocyte divides unequally producing larger and smaller secondary spermatocytes. The larger secondary spermatocyte undergoes a second maturation division, and produces two equal-sized spermatids which transform into functional spermatozoa. The smaller secondary spermatocyte, which has received fewer chromosomes is said to degenerate. Only two similar spermatozoa, consequently, are formed from a primary spermatocyte.

Von Baehr (1909 and 1912) states that he very rarely observed the development of the smaller secondary spermatocyte to the prophase of the second maturation division, but that it does not divide. Stevens (1909) says that in a preparation which has unfortunately been lost, the anaphase of the smaller secondary spermatocyte was seen, and that such stage may also be distinguished among the degenerating spermatocytes. Morgan (1915) states: "The small cell is left with two chromosomes and a small amount of cytoplasm. It never divides again, and later degenerates. Stevens was inclined to think that the small cell may

sometimes show a division figure, which subsequently fades away, but I have never seen a case of this kind."

In *Macrosiphum ambrosiæ* and *Neothomasia populicola* I have observed the late telophase of the smaller secondary spermatocytes. In *Stomaphis yanois,* moreover, I have found that the smaller secondary spermatocytes divide and form equal-sized spermatids which are much smaller than the larger spermatids. These smaller spermatids develop and reach the sustentacular cells with the developed larger spermatids; they, however, fail to attach to the sustentacular cells. Thus their development ceases; they, therefore, do not fully transform into spermatozoa, but retrogress and form spherical cells, which attach themselves to the epithelium of the cysts of the testes. A further account of this will appear in the following pages.

The work on the spermatogenesis of *Stomaphis yanois* was done in the Tokyo Higher Normal College, and the work on the other aphids has been done in the University of Chciago. The writer's thanks are due to Prof. F. R. Lillie and Prof. S. Yamanouchi, who gave him many suggestions and much help. The writer also wishes to thank Prof. A. Oka and Prof. U. Takakura for their kindness during his stay in Tokyo. For the identification of the aphids the writer is indebted to Dr. A. L. Quaintance and Dr. A. C. Baker.

II. Methods.

Both the males and parthenogenetic females were fixed in either strong Flemming's, Zenker's or a mixture of absolute alcohol one part, acetic acid one part, and saturated aqueous solution of corrosive sublimate two parts. Sections were cut 3, 5 and 10 micra in thickness; most of them, however, were cut 5 micra thick. They were stained with Heidenhain's iron-hematoxylin followed by eosin or borax carmin.

III. Stomaphis yanois.

1. *Primary Spermatocyte.*

Figs. 1 and 2 show the primary spermatocyte prophase. In Fig. 1, one of the chromosomes is formed, and in Fig. 2, the proc-

ess of the formation of the chromosomes is almost finished. There are ten chromosomes, five larger and five smaller, in the equatorial plate of the first spermatocyte division, and they are connected with one another by linin threads as is shown in Figs. 4 and 5. The side view of the mitotic figure shows centrosomes of about the same size agreeing with von Baehr's observation on *Aphis salicti* (Fig. 3).

In the anaphase unequal cell division is indicated. The larger and smaller daughter cells are connected by a bridge of cytoplasm, and elongated lagging chromosomes lie between the chromosomes passing to the daughter cells (Fig. 7). The lagging chromosomes do not show any tendency to go to the larger cell at this time, but after the nuclear membrane is formed, the lagging chromosomes enter the larger cell (Fig. 8). It is interesting to note that the size of the nuclear membrane is larger in the larger cell. The inequality of the size of the nuclei of the daughter cells, therefore, does not seem to be due to the unequal number of the chromosomes, but to an unequal quantity of cytoplasm. In a case where the two daughter cells were about equal the size of the nuclear membrane was about the same.

I have observed many cases in which the lagging chromosomes appear to be divided, but I doubt that this ever occurs. Morgan (1909) states: "That artificial conditions, such as handling or osmosis, might break such a delicate connection at this time is not at all improbable, and such an artificial result might give the impression that the accessory is actually divided. Moreover, if the bridge arches toward or away from the observer, the effect may be produced at certain focal levels of discontinuity between the ends of the lagging chromosomes, when none such exist."

The larger secondary spermatocyte receives eight divided and two lagging undivided chromosomes, and the smaller secondary spermatocyte receives eight divided chromosomes.

2. *Larger Secondary Spermatocyte.*

The larger secondary spermatocyte undergoes an equal second division without an intervening resting stage. The equatorial plate (Figs. 12 and 13) of the second division shows ten chro-

mosomes. In the first maturation division five of the ten chromosomes are larger and five of them are smaller, but in this case six are larger and four are smaller. The reason for this is discussed later on. As in the case of the first division, chromosomes are connected by linin threads. When the split chromosomes shift to the opposite poles interzonal fibers appear. In the first division the middle part of the two daughter cells becomes narrow and shows an appearance of a dumb-bell with the ends different in size. In this case, however, the middle part is broad, so that the interzonal fibers are separated (Fig. 15).

3. *Smaller Secondary Spermatocyte.*

The smaller secondary spermatocyte shows chromosomes in its nuclear cavity at the telophase of the first maturation division. It is not difficult to distinguish the smaller secondary spermatocyte as their diameter is hardly half that of the larger ones. The nucleus does not enter a resting stage. I have found in some cases two small bodies near the nuclear membrane (Fig. 9). These seem to be centrosomes, but I am unable to speak with certainty. The changes in preparation for the second division are similar to those of the larger spermatocyte.

The equatorial plate (Figs. 20 and 21) shows eight chromosomes as compared with ten chromosomes in the equatorial plate of the larger secondary spermatocyte. The cases which distinctly show eight chromosomes are rare; there can be little doubt, however, that this is the full number since there are ten chromosomes in the equatorial plate of the first maturation division, and two of them pass to the larger one as the lagging chromosomes. Four chromosomes are larger and the other four are smaller. There are five larger and five smaller chromosomes in the equatorial plate of the first division; the lagging chromosomes, therefore, must be a larger and a smaller chromosome. If all the chromosomes were to divide in the first division, five larger and five smaller chromosomes would appear in the equatorial plate of the second division. Two chromosomes, one larger and one smaller, lag and enter the larger cell without dividing. The smaller of the lagging chromosomes, consequently, becomes larger than the

other smaller chromosomes. This must be the reason why we see four smaller chromosomes in the larger secondary spermatocyte instead of five.

The side view of the metaphase of the smaller spermatocyte differs from that of the larger one in shape. It is more spindle-shaped (Fig. 22). Fibers are not seen distinctly in the preparations stained with iron hematoxylin. The two stained bodies on both sides of the chromosomes in the equatorial plate might be the centrosomes (Fig. 23). There are cases which show separated chromosomes, and cases which show massed chromosomes (Figs. 23–25). So far as my observation goes, in most cases the chromosomes seem to fuse soon after their splitting. The telophase does not show distinctly the interzonal fibers as in the case of the larger cell. Two equal smaller spermatids are produced after the division.

4. *Smaller Spermatid.*

The germ cells of each cyst of the testes are generally in about the same stage. When the spermatids are young the cysts are spherical in shape, but they elongate during the development of the spermatids. The young smaller spermatids (Fig. 28) have condensed nuclei, but the larger spermatids (Fig. 18) between which they lie have vesicular nuclei. These smaller and larger spermatids are seen all through the cyst. I have examined many cases in order to see whether the polarities of the larger and smaller spermatids are established with relation to the epithelium or not. Most of the young larger and smaller spermatids, which are seen near the epithelium, develop their tails toward the center of the cyst, but some of them may develop along the epithelium or develop their tails toward the epithelium. Those in the central part do not show any definite orientation, and in extreme cases spermatids existing side by side may show opposite directions. In cysts in which the larger spermatids are developed to the stage shown in Fig. 18, the orientation of the larger and smaller spermatids remains unchanged. In a little later stage, however, all the larger and smaller spermatids begin to orient in the same direction, and when the larger spermatids develop to the stage shown in Fig. 19, all are oriented in the same direction.

There must be an interaction, probably chemical, between the sustentacular cells and the larger and smaller spermatids. The larger and smaller spermatids in the outer part opposite the sustentacular cells and in the central part of the cyst generally move among the tails of the other spermatids toward the sustentacular cells, but those in the other parts move toward sustentacular cells along the epithelium.

Developed smaller spermatids (Fig. 31) are seen among the larger spermatids near the sustentacular cells, and do not show any inferiority to the larger spermatids in moving toward the cells. Before the nucleus of the larger spermatid shows marked differentiation the smaller spermatids have retreated a little towards the interior. In other words, well developed smaller spermatids approach towards the sustentacular cells, but do not attach to them. I have examined many smaller spermatids in order to see whether they develop apical parts. Figure 30 shows a developing smaller spermatid, which has a cone-shaped apical part. There is a developed smaller spermatid, which seems to have a well-developed apical part, but we cannot distinctly observe since it is seen in close contact with the tails of the larger spermatids. In most of the smaller spermatids, which have elongated tails, I have not, however, observed developed apical parts.

As to the interpretation of the cells identified as smaller spermatids, may they not be degenerating larger spermatids? So far as my observation goes the larger spermatids rarely degenerate; moreover, it is not hard to distinguish degenerating young larger spermatids from the smaller spermatids, since the former are not only much larger than the latter, but the nucleus of the larger spermatid becomes vesicular while the nucleus of the smaller spermatid is condensed. If the larger spermatids developed to the stage shown in Fig. 19 begin to degenerate, we can recognize them by the difference in the state of the nuclei. If the almost fully developed spermatids begin to degenerate, it is quite easy to tell them from the smaller spermatids, since they have very slender nuclei and the smaller spermatids, which are seen in the same cyst with them, have spherical nuclei. If degeneration of the larger spermatids should occur at the stage in which they

have condensed ovoid nuclei which elongate later, the criterion by which to distinguish them is their position, since when they have developed to such a stage, the smaller spermatids with condensed spherical nuclei have already left the epithelium.

The metaphase of the smaller secondary spermatocytes are seen among those of the larger secondary ones; I think, therefore, there is no doubt that the smaller secondary spermatocytes undergo the second division. More developed larger spermatids are seen with more developed smaller spermatids in the same cyst. We may conclude from these observations that the smaller spermatids develop with the larger spermatids.

I have observed cases where the larger and smaller spermatids are seen in the central part of the cyst, while the majority of spermatids have already reached the sustentacular cells. Such larger and smaller spermatids might fail to reach the sustentacular cells, since they have to move among the spermatids. The examination of the later stages, however, has shown that they succeed in reaching the sustentacular cells.

Figure 32 shows a smaller spermatid which is abnormally big and has a distinct axial filament. Ordinarily the smaller spermatids elongate similarly, but are more delicate. One of the most developed smaller spermatids is shown in Fig. 33. In such a stage their development comes to a standstill, and they begin to retrogress. They gradually retreat toward the tails of the larger spermatids. Their nuclei which are deeply stained with iron hematoxylin are seen among the tails of the larger spermatids in a somewhat regular position. Finally they pass out to the cavity of the cyst.

The smaller spermatids fail in attaching to the sustentacular cells; they cannot, consequently, get material for their further development. They have to live on their own substance. Their tails become shorter, and the cytoplasm around the nucleus increases (Fig. 34).

The forms shown in Fig. 35 are seen near the tail of the fully developed functional spermatozoa in the cavity of the cyst. We do not see such spermatids in the cavities of the cysts at the younger stages. These smaller spermatids still have elongated tails, but later transform into spherical cells which have a distinct cell

membrane (Fig. 39) and show a tendency to fuse with each other. There are some cells which have two or more condensed nuclei. These seem to be the products of the fusion of two or more smaller spermatids. Some of the retrogressed cells of the smaller spermatids attach to the epithelium, and on these cells other cells attach themselves; thus they form layers as shown in Fig. 38. In other cases they are irregularly attached to the epithelium. When they attach themselves to each other they show a polygonal shape.

A, b and *c* in Fig. 38 are parts of adjacent cysts, where fully developed spermatozoa occur though not shown in the figure. The cells occurring between the cysts are the retrogressed smaller spermatids produced in the cyst *c,* and the epithelium proper is very thin as seen between cysts *a* and *b*. As we see in the figure these cells are not equal in size. In some of them the nuclei are broken up and their fragments are seen scattered throughout the cells. The others still show condensed spherical nuclei. As stated already the larger spermatids rarely degenerate. These larger spermatids may become like the cells just mentioned. Though degenerating larger spermatids mingle among these cells, there is no criterion by which they may be distinguished from retrogressed cells of the smaller spermatids.

Some of these cells may be absorbed by the epithelial cells, but how far the absorption proceeds is at present undetermined. When these cells attach to the epithelium the functional spermatozoa are already fully developed. Afterwards the wall of the cyst ruptures, and these cells being deprived of their connection with the testis are destined to disappear. It is possible that they are extruded from the testis along with the spermatozoa. I have observed epithelial cells of the cyst and retrogressed cells of the smaller spermatids in some of the vasa deferentia. The sections of the testes of the old males show remarkable changes. Their walls are thickened and neither spermatozoa nor the cysts, which fill the young testes, can be seen.

IV. Neothomasia populicola and Macrosiphum ambrosiæ.

The testes of embryos of *Neothomasia populicola* and *Macrosiphum ambrosia* are in the early stages, but those of larvæ are

suitable for the purpose of studying the spermatocyte divisions. As is the case in other aphids, the primary spermatocytes of these aphids divide unequally, and the anaphase shows the lagging chromosomes. I have found in these aphids telophases of the second maturation division of the smaller secondary spermatocyte, but have observed no developing smaller spermatid; we may, therefore, conclude that the smaller secondary spermatocytes and the smaller spermatids of these aphids degenerate as in the cases of the aphids studied by Morgan, von Baehr and Stevens. In *Macrosiphum ambrosia* I observed cases in which all smaller secondary spermatocytes seemed to be dividing, but I will conclude in a succeeding paper whether all the smaller secondary spermatocytes divide or not.

In the cyst, where larger spermatids are already attached to the sustentacular cells, there are seen spermatids which look like the smaller spermatids of *Stomaphis yanois*. As stated above, since no development of the smaller spermatids was observed, they must be larger spermatids. In slightly younger cysts some spermatids are seen among the developed tails of other larger spermatids, which are about to attach to the sustentacular cells. Such spermatids probably have no chance of reaching the cells. I have found cases in which the larger spermatids are already attached to the cells, but some spermatids are seen among the ends of the tails of the larger spermatids. In other cysts spermatids with condensed nuclei are seen apart from the sustentacular cells, while others are attached to them.

As in the case of *Stomaphis yanois* young spermatids of these aphids change their orientation to the same direction; some spermatids, therefore, move to the sustentacular cells across the whole diameter of the cyst or reach the cells moving along the epithelium. If they move to the sustentacular cells along the epithelium, as most of the spermatids do, they may lose the chance to become attached to them. Developed spermatids have been found by the side of spermatids which are attached to the sustentacular cells and are developing. They were probably prevented from reaching the cells by other spermatids, and their development came to a standstill; they, consequently, show younger stages than the spermatids which are attached to the

cells. Since many spermatids are produced in the cysts, if they move to the sustentacular cells through the tails of other spermatids, they meet much resistance; they, therefore, might be unable to reach the cells.

The most conspicuous difference between the case of *Stomaphis yanois* and that of these aphids is the position of the retrogressing spermatids. In the former case the smaller spermatids approach the sustentacular cells, and then gradually retreat toward the tails of the larger spermatids; their position, consequently, is regular, having a relation to the development of the larger spermatids. In the latter case, however, the position of the retrogressing spermatids is irregular.

As in the case of *Stomaphis yanois* retrogressed spherical cells are seen in the cyst with fully developed spermatozoa. These cells attach themselves to the epithelium of the cysts and have the same fate as the retrogressed cells of the smaller spermatids of *Stomaphis yanois*.

V. Review.

According to Meves and others, one of the secondary spermatocytes of the honey bee is much smaller than the other, and receives no chromosomes; it, consequently, degenerates after some time. The larger secondary spermatocyte, moreover, divides unequally in the second spermatocyte division. The chromosomes divide this time, and there are produced larger and smaller spermatids. The larger spermatids differentiate into functional spermatozoa. The smaller spermatids also undergo some differentiation which, however, comes to a standstill at a late stage and then they degenerate without transforming into functional spermatozoa. The smaller spermatid of *Stomaphis yanois* resembles that of the honey bee in some respects. Both of them are much smaller than the larger spermatids, but judging from the Meves' drawings, the difference in size between the larger and the smaller spermatids is greater in the honey bee than in the aphid. They both develop to some extent, but do not transform into functional spermatozoa. Meves does not state what kind of changes occurs in the degenerating smaller spermatids of the honey bee; I am, therefore, unable to compare their later stages with those of the smaller spermatids of *Stomaphis yanois*.

The most conspicuous difference between the smaller spermatids of the honey bee and this aphid is seen in the nuclei. The nucleus of the smaller spermatid of the honey bee returns to a resting stage, and differentiates similar to that of the larger spermatid. The nucleus of the smaller spermatid of this aphid, however, becomes condensed after the second spermatocyte division, and remains in the same state, although the cytoplasm shows changes similar to those of the larger spermatid. This may be caused by the absence of the lagging chromosomes in the smaller spermatids, while in the honey bee the smaller spermatids have the same number of chromosomes as the larger spermatids.

Whitney (1918) mentions that the normal and rudimentary spermatozoa have been found in considerable number of rotifers. In his paper of 1917 he says that the functional spermatozoa are identical in their power of determining the sex of the individual that develops from a fertilized egg, since after a functional spermatozoon has fertilized a parthenogenic male egg, the egg always develops into a female individual.

In the case of these rotifers, according to Whitney, the chromosomes divide in the first spermatocyte division. One half of the secondary spermatocytes divide and form the normal spermatids. The remaining half of the secondary spermatocytes, contrary to the case of the smaller secondary spermatocyte of *Stomaphis yanois,* do not divide, but develop directly into the degenerate spermatozoa. The spermatocytes destined to degenerate are smaller than the others, and their development into the complete rudimentary spermatozoa is strikingly different from the development of the normal spermatids.

Whitney ('18) says that as all the fertilized eggs in both phylloxerans and rotifers develop into female young, it seems safe to conclude, as Morgan has already concluded, that the degenerate sperm cells are the male-determining ones and that the normal sperm cells are the female-determining ones.

Stevens (1905) found many degenerate spermatozoa in *Blattella germanica.* She states that the distribution and varying number of these degenerate spermatozoa make it impossible to interpret their condition as due to the absence of the accessory chromosome as Miss Wallace does in the spider, and that the only

probable explanation seems to lie in the imperfect mitosis. She detected no evidence of degeneracy among the young spermatids.

VI. Summary.

1. In *Stomaphis yanois* the smaller secondary spermatocytes divide, and develop to some extent, but retrogress to spherical cells.

2. In *Neothomasia populicola* and *Macrosiphum ambrosia*, cases of division of the smaller secondary spermatocytes were found, but no developing smaller spermatids were observed.

3. In *Neothomasia populicola* and *Macrosiphum ambrosia* spherical cells like those in *Stomaphis yanois* were found in the cysts containing spermatozoa. These were identified as retrogressed larger spermatids.

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EXPLANATION OF PLATES.

All of the drawings were made with the aid of camera lucida. Figs. 1 to 15 were drawn with a Leitz 1/16 oil immersion objective and a Zeiss compensating ocular 18. Figs. 16 to 37, except Fig. 33, were drawn with a Leitz 1/16 oil immersion objective and a Leitz ocular 5. Fig. 33 was drawn with a Leitz 1/16 oil immersion and a Leitz ocular 4. Fig. 38 was drawn with a Leitz 1/16 oil immersion objective and a Leitz ocular 3. All figures from *Stomaphis yanois*.

PLATE I.

FIGS. 1 AND 2. Primary spermatocytes, prophase.
FIGS. 3, 4, 5 AND 6. Primary spermatocytes, metaphase.
FIG. 7. Primary spermatocyte, anaphase.
FIGS. 8 AND 9. Primary spermatocytes, telophase.

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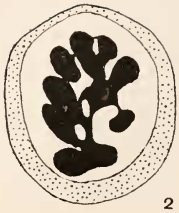

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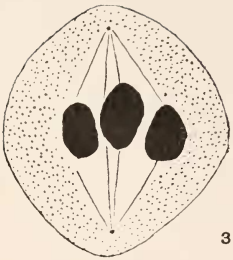

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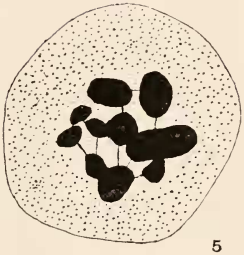

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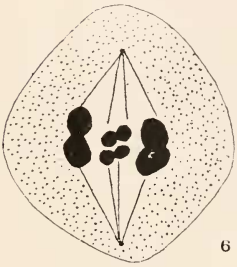

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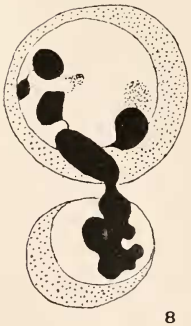

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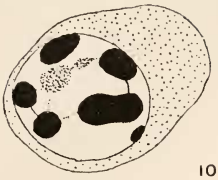
Plate II.

Fig. 10. Larger secondary spermatocytes, prophase.
Figs. 11, 12 and 13. Larger secondary spermatocyte, metaphase.
Figs. 14 and 15. Larger secondary spermatocytes, anaphase.
Fig. 16. Larger secondary spermatocyte, telophase.
Figs. 17, 18 and 19. Larger spermatids.



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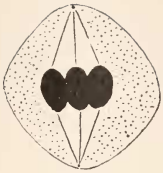

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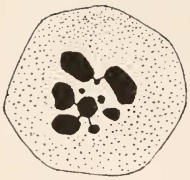

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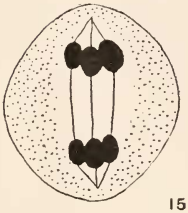

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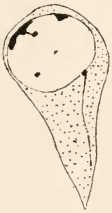

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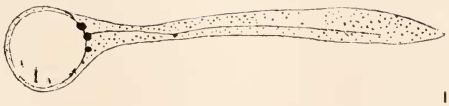

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Plate III.

Figs. 20, 21, 22, 23 and 24. Smaller secondary spermatocytes, metaphase.
Figs. 25 and 26. Smaller secondary spermatocytes, anaphase.
Fig. 27. Smaller secondary spermatocyte, telophase.
Figs. 28, 29, 30 and 31. Smaller spermatids.

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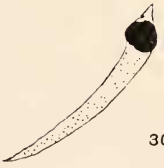

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Plate IV.

Figs. 32 and 33. Smaller spermatids.
Figs. 34, 35 and 36. Retrogressing smaller spermatids.
Fig. 37. Retrogressed cell of the smaller spermatid.
Fig. 38. Layers of the retrogressed cells.

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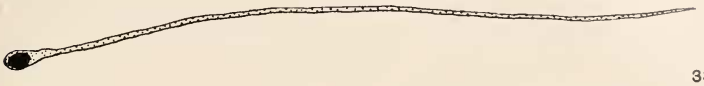

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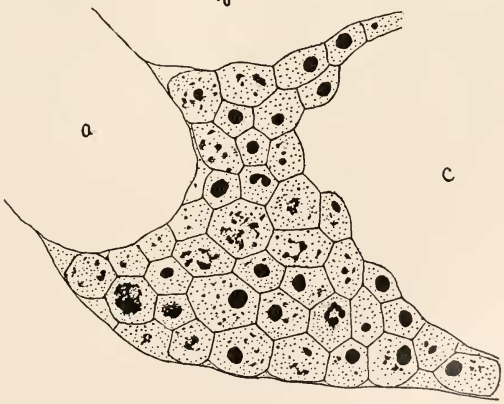

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